SEROLOGICAL STUDIES ON GENOTYPIC RELATIONSHIP AMONG RICE VARIETIES(1)

by

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Introduction

The genetic variability in a segregating population may suggest the degree of genetic diversity between the parents. An efficient method for asaying the genetic relationship among genotypes would be valuable for predicting the best cross combinations.

Modern genetics revealed that DNA controls protein synthesis through a series of reactions involving m-RNA and ribosome. Therefore the diversity of proteins responsible for this biosynthesis in different genotypes might provide an index for genetic diversity.

The serological technique was first used by Koworaki (1901) for identification of proteins in plants. He showed that the sera from rabbit with heat resistant wheat albumose reacted strongly with wheat albumose but weakly reacted or not reacted with the albumoses of rye, barley, oats and peas. Nelson and Birkeland (1929) were the first who serologically measured genetic relationships between varieties of a species wheat. Urano (1951) found that two lines of maize from the same S10 plant gave stronger serological reaction thanlines from the same S3 or S5 plant. Kleese and Frey (1964) studied different serological methods to determine which would best predict the genetic relationships among seven oat varieties and among four corn inbreds. They found that the serological data was correlated with genetic relationships estimated from within-cross variance components of oat lines and with the relative vigor of the single crosses between corn inbreds.

Kato and Maruyama (1928) used the precipitation technique and observed differentiation among Japanese, Chinese, Korean and Taiwanese rice varieties. Miu and Yao (1941) could also distinquish rice varieties by the serological relationship. The present study was undertaken to look into the genetic relationships between rice varieties belonging to different varietal groups by the precipitation reaction and immuno-diffusion technique.

materials and Methods

Two rice varieties(*Oryza sative L.*) from Taiwan, *i.e.*, Taichung (n) 1 (*indica* type) and Taichung 65 (japonica type), and one Japanese variety Nakamura, were used

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to immunize rabbits and to produce antisera. In addtion, more than 30 varieties from various sources listed in Table 1· were used for estimation of serological reactions. Each weighed about 2 Kg, were used as the immunization animal.

Table 1. List of rice strains used for serological test.

Variety No.	Variety name	Grain size	Phenol reaction	Type	Source
101	Taichung (n) 1	S	+	*indica*	Taiwan
102	Taichung (sen)2	S	+	〃	〃
104	Pai-mi-fen	S	+	〃	〃
105	Pei-ku	S	+	〃	〃
120	Taichung 65	S	−	*japonica*	〃
123	Taichung 171	S	−	〃	〃
127	Chicnung 242	S	−	〃	〃
129	Pai-kan-tao	S	−	〃	〃
130	Tamanishiki	S	−	〃	〃
151	Century 231	L	−	long grain	USA
152	P.I. 245717	L	−	〃	〃
153	Fortuna	L	−	〃	〃
154	Bluebonnet 50	L	−	〃	〃
155	Toro	L	−	〃	〃
156	Starbonnet	L	−	〃	〃
158	Belle Patna	L	−	〃	〃
159	Bleubelle	L	−	〃	〃
162	H 4	M	+	medium grain	〃
163	Zenith	M	−	〃	〃
164	Nova 66	M	−	〃	〃
165	Nato	M	−	〃	〃
166	Saturn	M	−	〃	〃
167	C.I. 9593	M	−	〃	〃
168	Eary Prolific	M	−	〃	〃
169	C.I. 9628	M	−	〃	〃
170	Lacrose	S	−	short grain	〃
171	C.I. 9442x57 F1 6067	S	−	〃	〃
172	Colusa (La.)	S	−	〃	〃
173	Colusa (Calf.)	S	−	〃	〃
174	Rexaro	L	−	long grain	〃

175	Gulfrose	M	−	medium grain	〃
177	C.I. 8670	M	+	〃	〃
J301	Yoshino 1	S	−	*japonica*	Japan
J302	Nakamura	S	−	〃	〃
J303	Aikawa	S	−	〃	〃
J306	Shinriki (3055)	S	−	〃	〃
J307	Shinrik (3052)	S	−	〃	〃
J310	Lun-sen-tao	S	−	〃	〃
4901	HT 19		+	*indica*	India
8263	Padi ladangase peolo komok		+	〃	Indonesia

5g of the brown rice grains were ground with a STUR-DEE MITEY-MILL as fine as pssible. The rice powder was pre-extracted twice at 4°C, with ether to remove lipids. The defatted rice powder was air dried at 4°C Then 50 ml. of 0.85% sodium chloride was added and mixed well for 10 hours at 4°C. The extract was centrifuged in a reqrigerated condition at 1.700 rpm for 30 minutes. Clear supernatant was taken. filtered through cheese cloth to remove floating segments and stored at 4°C. This was ready for injection.

Prior to injection, 10ml. of blood was drawn from the heart of each rabbit as the control. Intravenous injection through the ear vein with 1 ml. of the antigen was administrated initially. The quantity of antigen injected was increased to 1.5 ml. for the second injectionone week later. From the 3rd to 7th injections, the quantity of antigen was increased to 2 ml at a three-day interval. Three ml. to each rabbit was given at the last injection and 15.5 ml. in total.

The immunization period was one month. Longer period of immunization was avoided, because there was the possibility of losing antibody specificity. One month after the last injection. 50 ml. of blood was taken from each rabbit by cadilac puncture. The rabits were fasted 13 hours previous to bleeding to reduce serum lipid content in order not to make the serum turbid.

The sample of blood was placed in clean test tubes· allowed to coagulate, and was then incubated for four hours at 35°C to allow the clots and serum to separate. Then the separeted serum was poured into plastic tubes (10 ml.) and was centrifuged at 3,000 rpm for five minutes. After the removal of the free cells, it was frozen and stored at 0°C. About 20 ml. of serum was yielded from 40 ml. of flesh blood.

Precipitation reaction

The entire range of antigen-antiserum reaction from antigen excess to antibody excess was assayed by series of doubling antigen dilutions (*i.e.* 1, $\frac{1}{2}$, $\frac{1}{4}$. etc.) and constant amount of antiserum. The quantities of antiserum and antigen solutions used for each test were 0.05 and o.25 ml. respectively. The precipitin reaction was measured by the apparent turbidity of the antigen-antibody system. The magnitude of reacton was recorded accor-

ding to four levels· *i.e.*, completely precipitated (+++), inciomplete (++), no precipitation but apparent turbidity of the solution (+) and no reaction (−).

Most tubse in whih reaction occured reached the maxmum turbidity within one to two hours The reading of reactions was performed at room temperatures. The diluent for assaying rabbit antisera was 0.85% sodium chloride solution.

Double Diffusion Gel Tachnique

The gel diffusion technique described by Ouchterlony (1948; 1949) was employed to study qualitatively antigen differences among rice varieties. Agar medium was prepared by dissolving Oxoid lonagar No.2 (Consolidated Laboratories· inc., Chicago Heights, Illinois,) at a concentration of 0.8% in 0.1 M phosphate buffered physiological saline with 1.0% sodium aside added as a preservative. Five ml. of agar (a 2.5 mm layer) was poured into petri dishes five cm in diameter. After the agar was set, a center well and six surounding wells were cut using a special mold.

Undiluted antisera were placed in the center well and antigens of varieties to be tested were placed in the six surounding wells. The plates were placed at room temperatures in a moisture saturated at mosphore for 48 to 72 hours. Observations on the pattern of precipitated bands were made 48 hours after antigen–antibody reaction. The typical patterns were photographed.

Experimental Results

1. Identifion of genetic differences between rice varieties by precipitation reaction.

It is known that when certain antigen (protein) is injected intravenously into an animal, it will stimulate to produce antibodies specific to that protein. When this specific antibody is titred with another antigen, if it is identical in protein specificity, a precipitation reaction will occur. The precipitation reaction expressed by turbidity then can be a qualitative or quantitative measure of genetic relationship.

In the present study, turbidity was measured qualitativery. In the mixture of antigen-antibody solution, if precipitation occurs within a short time even in dilute solution, there is a close relationship between the two varieties. If the precipitation reaction is incomplete, it indicates that the two varieties are distantly related. If there were no relationship between two varieties in terms of protein specificity, no reaction would occur. According to this principle, the antiserum of Taichung (n) 1, (*indica type*) was titred with the antigens of other varieties. The results are given in Tabel 2

Tgble 2. Degree of precipitation reaction from Taichung (n) 1 antiserum against antigens of other varieties

As shown in Table 2, the homologus reaction of Taichung (n) 1 began to express itself 15 minutts after mixing antigen and antibody. 30 minutes later, it began to precipitate gradually increasing its magnitude and completed precipitation in 60 minutes. Taichung (sen) 2 and Pai-mi-fen showed a positive reaction showing that these two varieties were serologically identical with Taichung (n) 1.

Ponlai varieties, Taichung 65 Taichung 171, Chianung 242 and Pai-kan-tao as well as Japanese varieties, Tamanishiki, Nakamura and Shinriki, showed no reaction to the

Antigens / Dilution	1.0				0.5				0.25				0.125				CK
	15*	30	60	120	15	30	60	120	15	30	60	120	15	30	60	120	
Taichung (n)1	+	++	++	+++	+	++	+++	+++	+	++	+++	+++	+	++	+++	+++	−
Taichung (sen) 2	+	+	++	+++	+	+	++	+++	+	+	++	++	+	+	++	++	−
Pai-mi-fen	−	+	++	+++	−	+	++	+++	−	−	++	+++	−	−	++	+++	−
Taichung 65	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Taichung 171	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Chianung 242	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Pai-kan-tao	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tamanishiki	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Shinriki	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P.I. 245717	+	++	++	+++	+	+	++	+++	+	+	++	+++	−	+	++	++	−
Fortuna	−	−	+	++	−	−	+	++	−	−	+	++	−	−	+	++	−
Bluebonnet 50	−	−	+	++	−	−	+	++	−	−	+	++	−	−	+	++	−
Toro	−	−	+	++	−	−	+	++	−	−	+	++	−	−	+	+	−
Rexaro	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−
Eary Prolific	−	−	+	+	−	−	+	+	−	−	+	++	−	−	++	++	−
Saturn	−	+	+	++	−	−	+	++	−	−	+	++	−	−	+	+	−
H 4	−	+	+	++	−	+	+	++	−	+	+	++	−	−	+	+	−
C. I. 9593	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Colusa	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
C.I.9442x57 F1 6067	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lacrose	−	−	+	+	−	−	+	++	−	−	+	++	−	−	−	++	−
Gulfrose	−	+	+	+	−	−	+	+	−	−	+	+	−	−	−	+	−
HT 19	+	++	++	+++	+	++	++	+++	+	++	++	+++	+	++	+++	+++	−
Padi ladangase	+	+	+	++	+	+	++	+++	+	++	++	+++	+	++	++	+++	−
Poeloe Komok																	

*minutes after the antigen and antibody solution were mixed

antiserum of Taiching (n) 1. This shows that these varieties are not related serologically with Taichung (n) 1. US long grain varieties P.I. 245717, Fortuna, Bluebonnet 50, Toro and Rexaro showed a positive reaction to the antiserum of Taichung (n) 1, but the magnititude of reaction differed among them. Fortuna, Bluebonnet 50 and Toro began to react in 60 minutes and in 120 minutes with Rexaro, after the two solutions were mixed. This shows that these varieties are rather distantly related with Taichung (n) 1, probably because of a small number of reacting sites in protein structure. Medium grain varieties H4 and C.I. 9593, short grain varieties colusa and C.I. 9442 x57 F1 6067 were not found to

be related to Taichung (n) 1, as they showed negative reaction. The *indica* varieties from India and Indonesia, NT19, padi ladangase poeloe komok showed a positive reaction to the antiserum of Taichung (n) 1.

Table 3. Degree of precipitation reaction from Nakamura antiserum against antigens of other varieties.

Antigens	1.0				0.5				0.25				0.125				CK
Dilution	15	30	60	120	15	30	60	120	15	30	60	120	15	30	60	120	
Taichung(n) 1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Taichung (sen) 2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Chianung 242	+	+	++	+++	+	+	++	+++	+	+	++	++	−	+	++	++	−
Century 231	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Eary prolific	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lacrose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P.I. 245717	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bluebonnet 50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bluebelle	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H 4	+	+	++	++	+	+	++	++	−	+	++	++	−	−	+	++	−
Nova 66	−	−	+	+	−	−	+	++	−	−	+	+	−	−	+	+	−
Nato	−	+	+	++	−	−	+	++	−	−	+	++	−	−	+	++	−
C.I. 9442x57F16067	−	−	+	++	−	−	−	+	−	−	−	+	−	−	−	+	−
Colusa	−	−	+	+	−	−	++	++	−	−	+	++	−	−	−	+	−
Rexaro	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Gulfrose	+	+	+	+	+	+	+	+	−	−	+	+	−	−	−	+	−

*minutes after the antigen and antibody solution were mixed

Table 4. Degree of precipitation reatcion from Taichung 65 antiserm against antigens of other varieties.

Antigens	1.0				0.5				0.25				0.125				CK
Dilution	15	30	60	120	15	30	60	120	15	30	60	120	15	30	60	120	
Yoshino 1	+	++	++	+++	+	++	++	+++	+	++	++	+++	+	+	+	++	−
P.I. 245717	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bluebonnet 50	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Toro	−	−	+	+	−	−	+	+	−	−	+	+	−	−	−	−	−
Starbonnet	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Belle patna	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bluebelle	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H 4	−	−	+	++	−	−	++	++	−	−	++	++	−	−	++	++	−
Nova 66	−	−	+	+	−	−	+	+		−	−	−	−	−	−	−	−

*minutse after the antigen and antibody solution were mixed

The reactions of antiserum from Nakamura (*japonica*) with the antigens of other varieties are given in Table 3. As shown in table, a majority of varieties which showed positive reactions in Table 2 were negative while those showing negative reactions in Table 2 were positive with the exception of Gulfrose and H4 which showed a positive reaction to both of the antisera.

In Table 4, the reactions of antiserum from Taichung 65, a typical Ponlai (*japonica* type) variety of Taiwan, against other varieties are shown. As shown in Table 4, a positive reaction was seen when the antigen of Yoshino 1 was titred against the antiserum of Taichung 65. The reactions of Blue belle, H4 and Nova 66 were consistent with those of Nakamura given in Table 3, but Toro differed showing a positive reaction with both the *indica* (Taichung (n) 1 and *japonica* (Taichung 65) types of antisera. This reason remained unelucidated. They probably belong to an intermediate type between the *indica* and *japonica* types, possessing reacting sites of both groups.

As shown in Tables 2, 3, and 4, both the positive and negative reactions to the *indica* and *japonica* type antisera were found in US long (Toro) medium (H4, Gulfrose) grain groups. Probably, we can not determine the US long grain varieties to be *indica* types and the US medium and short grain varieties to be *japonica* types. because no clear serological relationship was found among the long, medium or short grain variety groups.

2. Identification of genetic relationships among rice varieties by gel-diffusion method

Antibodies formed by the stimulates of a given antigen usually do not react with other antigens unless those are closely reacted. This also true often reaction of one antigen with antibodies. By gatigen analysis the serological approach of ten permits more sensitive and specific identification of unknown substances than other biological or chemical methods.

In the present study, the constant antibodies in the center well and different antigens in the surrounding wells were placed on a gel plate. The precipitates which appear as bands in the region between the two sources of diffusion were recorded. The pattern of precipitates changed as the time of reaction prolonged.

Figures 1-3 show precipitates by the gel-diffusion technique of antisera Taichung 65 (*japonica* type) and Taichung (n) 1 (*indica* type) against the antigens of other varieties.

As shown in Fig. 1, one precipitated band appeared when the anti-serum of Taichung 65 cross-reacted with the antigens of Taiehung 65, Shinriki, Saturn and C.I. 9593. This indicated that these varieties are closely related with Taichung 65 serologically. Since Taichung 65 belongs to the *japonica* type, those of cross-reacted ones could be identified to belong to the same group. It is noted further that the precipitated band produced between antiserum Taichung 65 and antigen Saturn, and that produced between antiserum Taichung 65 and C.I. 9593 were connected each other end to end showing that proteins of these two varieties are identical. In contrast, no precipitated band occured between the antiserum of Taichung 65 (*japonica*) and the antigens of Taichung (n) 1 (*indica*) and Taichung (sen) 2 (*jndica*), showing that there is no serological relationship between the *japonica* and *indica* groups. These results are consistent with the results shown in Table 4. It is inferred that unknown rice varieties may be classified into groups preferably *indica* and *japonjca* sub-groups conveniently by serological analysis.

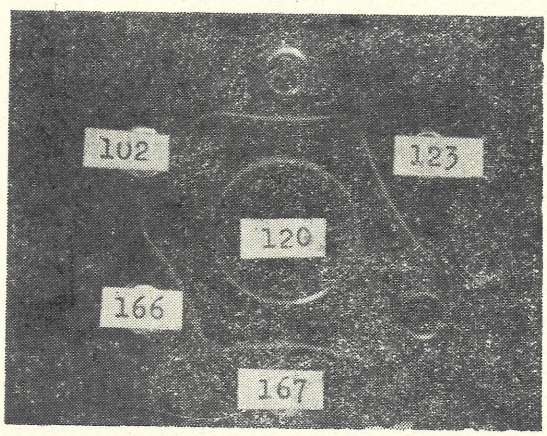

Fig. 1: Precipitation bands from gel-diffusion method with Taichung 65 antiserum against antigens from other rice cultviarss.

101 Taichung (n) 1 (*indica* type)
102 Taichung (sen)2
120 Taichung 65 (*japonica* type)
123 Taichung 171
166 Saturn US medium grain
167 C.I. 9593
J306 Shinriki *japonica* type
(Photographed at 48 hours)

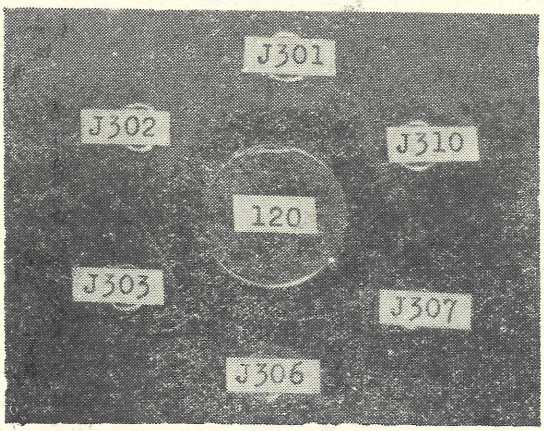

Fig. 2: Precipitation bands from gel-diffut ion method with Taichung 65 ant iserum against antigens from Japanese rice cultivars.

120 Taichung 65
J301 Yoshino 1
J302 Nakamura
J303 Aikawa
J306 Shinriki 3055
J307 Shinriki 3052
J310 Lun-sen-tao
(photographed at 96 hours)

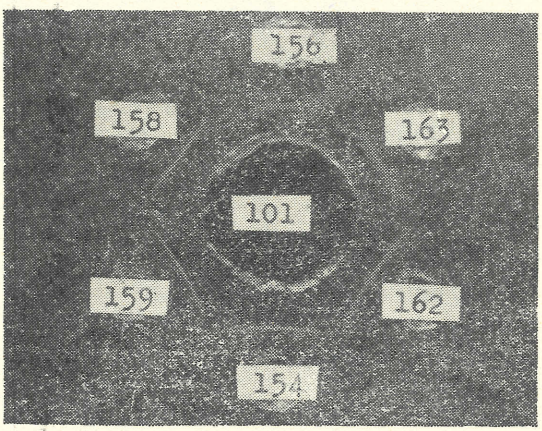

Fig. 3: Precipitation bands from gel-diffu sion method with Taichung (n) 1 antiserum against antigens from 6 American rice cultivars.

101 Taichung native 1 *indica* type
154 Bluebonnet (50 US long grain)
156 Starbonnet (〃)
158 Bella Patna (〃)
159 Bluebelle (〃)
162 H 4 US medium grain
163 Zenith (〃)
(Photographed at 48 htours)

Fig. 2 shows cross reactions within the *japonjca* group, using antiserum Taichung 65 titred with proteins of Yoshino 1 (J301), Nakamura (J302), Aikawa (J303) Shinriki (J307) and Lun-sen-tao (J310). All varieties showed an identical reaction with Taichung

65. It is further noticed that one additional minor band appeared in addition to the identical major bands between the antiserum of Taichung 65 and the antigens (proteins) of Yoshino 1. Nakamura, Lun-sen-tao and Shinriki showingt that there is a close relationship between these varieties.

In order to know the genetic relationship of US varieties, they were cross-reacted with the antisera of Taichung (n) 1 and Taichung 65. Fig. 3 shows an example. As shown in the figures, four US long grain (154, 156, 15 8,159) and two medium grain (164, 163) varieties cross-reacted with the *indica* type antiserum of Taichung (n) 1 indicating that they were serogically in the same group.

According to the above mentioned criteria, the genetic relationships among rice varieties obtained by serological tests are summarized in Table 5.

Table 5. Grouping of rice cultivars based on patterns of precipitated bands when antisera of Taichung (n) 1 and Taichung 65 were used with gel-diffusion method.

Class	Cultivars	
Precipitated bands with Taichung(n) 1 anti-serum only	Taichung (n) 1	C. I. 9628
	Taichung (sen) 2	Laorose
	Pai-mi-fen	
	Bluebonnet 50	
	Starbonnet	H 4
	Belle Patna	Zenith
	Bluebelle	Nato
	Early Prolific	Rearo
		Nova 66
Precipitated bands with Taichugn 65 antiserum only	Pai-kan-tao	Aikawa
	C.I. 9593	Shinriki (3055)
	C. I. 9442x57 F1 6067	Shinriki (3052)
	Yoshinol	Lun-sen-tao
	Nakamura	
Precipitated bands with antisera from both Taichung (n)1 and Taichung 65	Saturn	

As shown in Table 5, the antigens (varieties) which reacted with the antibody of Taichung (n) 1, in most cases do not react with the antibody of Taicnung 65; the same is true in the case of antibody Taichung 65. As stated before, varieties of a group can be further classified into identical, partly identical and different groups accorrding to their protein similarity. This classification is given in Table 6. As the data in Table 6 show, four closely related sub-groups within the *indica* group and these sub-groups within the *japonica* groups were distinguished.

Table 6. Subgroups of rice cultivars within *indica* and *japonica* types based on precipitin reactions with gel-diffusion procedure.

Type	Subgroups of cultivars	Cultivars that fit no subgroup
indica	1. Taichung (n)1 Taichung (sen) 2 Pai-mi-fen	Starbonnet Belle Patna Toro
	2. Bluebonnet 50 H 4 Zenith	Laorose Gulfrose C. I. 8670
	3. Nova 66 Nato Early Prolific C. I. 9628	
japonica	1. Yoshino 1 Nakamura Aikawa Shinriki (3055) Shinriki (3052) Lun-sen-tao	Taichung 171 Pai-kan-tao
	2. Saturn C. I. 9593.	

DISCUSSION

Serology has been employed as a technique in plant systematics for *Magnoliaceae* (Johnson, 1954), *Cucurbitaceae* (Baum, 1954), *Solanaceae* (Hammond, 1955) and forage grasses (Fairbrothers and Johnson, 1959). Gell *et al* (1959)used immunoelectrophoresis in their taxonomic study of *Solanum*. Relationships among species suggested by serological techniques generally have been similar to those postulated from morrphological and cytological data. Hall (1959),using electrophoresis, found wheat and rye had some proteins in common and some different. A rye–wheat hybrid had all the wheat proteins and all but one of the rye proteins. Kato and Murayama (1928) used the precipitin technique to differentiate *indica* and *japonica* rice cultivars.

Herein, we were able to separate rice cultivars not only into *indica* and *japonica* types, but also we could place many cultivars into subgroups within each type on the basis of the gel–diffusion data. Now, the occurrence of the precipitin reaction is due to some type of compatibility or comparability between antigen and antibody. Our data suggest that a major portion of the antigens extracted from the rice grain by physiological saline solution were related to the differentiation of the *japonica* vs. *indica* traits since *japonica* incited antiserum (e.g., to Taichung 65) gave no apparent precipitation when titred with *indica* anti gens (e.g.r om Taichung (n) 1). we do not know, of course, whether the extracted antigens were proteins of direct genetic mediation (i.e., DNA RNA protein) or whethe r they may have been secondary or even storage proteins.

Indica and *japonica* types of rice both belong to the species *Oryza sativa*, but Jodon (1955) postulates that they have been grown in different environments so long that much accumulated genetic divergance probably has occurred between them. The *indica* type has evolved in the tropical area, i.e., India, Indonesia, The Philippines, South China, whereas the *japonica* type probably evolved in a more northern sub–tropical area, i. e.,

Japan, Korea, and North China. Trait differences between the two rice types are quite numerous. For example *japonica* rice cultivars are short grained, photoperiod sensitive, short and they tiller freely and respond well to heavy fertilization, whereas *indica* rice cultivars are medium to long grained and tolerate adverse environmental conditions (Leonard and Martin, 1963). Kuang and Tu(1949) found a mean fertility of F1's between *indica* and *japonica* cultivars of 9.3%, wheres crosses within types had a mean fertility of 77.4%. Even though *indica* x *japonica* crosses are largely sterile, some fertile progenies can be selected from most crosses (Jones,1930). Many of the current American cultivars were derived from just such crosses. Our serological evidence suggested that some of these cultivars had both *indica* and *japonica* antigens. The degrees of reaction between antigens from these intermediate cultivars and antigens against *indica* or *japonica* cultivars were dependent very much on the specific genotype tested.

It appears that the serological method could be useful to classify rice cultivars into *japonica* and *indica* types. And with the gel-diffusion technique even identical and non-identical reaction bands can be differentiated. The future importance of such a classific ation system probably is not predictable. Very likely, not all genetic differences between *indica* and *japonica* types are differentiated, so even. distinguishing of apparently iden tical and non-identical cultivars via gel-diffusion may not necessarily tell whether crosses involving one *japonica* and one *indica* and two "identical" indica cultivars will provide comparable F_2 offspring arrays. Certainly, future serological research with plant antigens should concentrare on isogenic lines to determine whether minute and all genetic differ ences can be detected immunologically. The method in its present state of development may be useful in cultivar identification.

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Summary

Three rice cultivars, Taichung (n) 1 (*indica* type) and Taichung 65 and Nakamura (*japonica* types) , were used to immunize rabbits to obtain antisera. Another 37 rice cultivars from various countries were used as antigen sources for studies of antigen-antibody reactions between the two groups of cultivars.

When the antisera incited by immunization with seed protein of *japonica* type cultivars were titred with antigens from known *japonica* type cultivars, a positive precip itation reaction occured, but no reaction was obtained with antigens from *indica* type cultivars. In a few cases antigens from a cultivar reacted with both *indica* and *japonica*

type antisera. The antigens from these intermediate types may have had reating sites for both types of antisera or the antigen complex may have contained two antigen types, one for *indica* and one for *japonica*. These intermediate cultivars that appeared to have both *indica* and *japonica* antigens may have been selected from *japonica* x *indica* crosses.

The gel-diffusion technique showed rice cultivars within *indica* or within *japonica* could be grouped into subgroups on the basis of the pattern of their precipitated bands. There was no relationship among American rice cultivar for grain length, i.e., long, medium or short *japonica* and *indica* type.

The serological method would be useful for assigning rice cultivars to *japonica* or *indica* types and perhaps to subgroups within these types. Whether it would be useful to rice breeders to pre dictdesirable crosses must await additional study,

利用血清反應法之稻品種間遺傳型關係之研究

謝 順 景

用臺中在來一號（印度型）及臺中65號與中村（日本型）稻品種之谷粒蛋白質爲抗原（antigen），注射於家兎之靜脈內，以製造抗血清（antisera）。另外將來自世界各國的稻品種蛋白質作爲抗原，進行抗原一抗體反應試驗以測定不同品種間之遺傳型。

當由注射日本型稻蛋白質後所產生的抗血清與其他日本型稻蛋白質抗原混合，則發生沉降反應（Precipitation reaction），但與印度型稻抗原混合則無反應發生。有的抗原能同時對日本及印度二型抗體發生反應。此種抗原可能具有能與日本型及印度型二種抗原反應的兩個反應位置（reacting sites）。此種中間性品種可能來自日本型及印度型之雜交後代。

因寒天擴散（gel-diffusion）技術測定結果認爲可以根據不同抗原及抗體間所產生沉降帶（precipitated band）之樣式而將日本型稻或印度型稻品種再分類爲小羣（sub-group）。本研究結果顯示美國稻之長、中、及短粒型分類與日本型、印度型分類法並無關係存在。本研究認爲血清反應法可以用來預測稻之不同遺傳型，但在雜交育種上之實際應用尙需進一步的研究。